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Mitochondrial DNA Indicates that Extirpated *Ovis canadensis texianus* was a Member of the Desert Bighorn Sheep Complex

Emily A. Wright, Michael R. Buchalski, and Robert D. Bradley

Abstract

Described in 1912, *Ovis canadensis texianus* historically occupied 16 mountain ranges in the Trans-Pecos ecoregion of Texas but was extirpated in the early 1960s, presumably due to a combination of overharvesting, competition with livestock, disease, and predation. Although the genetic composition of *O. c. texianus* was unknown, restocking efforts depended on translocating desert bighorn sheep (DBS) from Arizona, Nevada, Utah, and Mexico. We used two mitochondrial markers (Cytochrome-*b*, *Cyt*b; displacement loop, D-loop) to determine the subspecific status of, and characterize genetic variation of, known pre-extirpation individuals representative of *O. c. texianus* and to compare haplotype profiles to individuals representative of *O. c. nelsoni*, including formerly recognized *O. c. cremnobates* and *O. c. mexicana*. Phylogenetic analyses, using *Cyt*b (n = 8) and D-loop (n = 19) datasets, indicated that most of the pre-1960 Texas bighorn sheep were genetically similar to individuals representative of DBS. However, using the D-loop dataset, two individuals from the Guadalupe Mountains collected in 1901–1902 were genetically similar to Rocky Mountain bighorn sheep (*O. c. canadensis*), necessitating further investigation to determine if Rocky Mountain bighorn sheep were present in the Guadalupe Mountains or surrounding areas in the Trans-Pecos ecoregion of Texas in the early 1900s. Although *O. c. texianus* originally were reported to be morphologically distinct from other bighorn sheep subspecies, levels of genetic divergence from current populations of DBS were low (0.14%), indicating a close genetic association. Further, individuals representing *O. c. texianus* and other DBS representative of *O. c. cremnobates*, *O. c. mexicana*, and *O. c. nelsoni* formed a single clade with no supported subclusters indicative of subspecific delineation, calling into question the validity of *O. c. texianus* as a subspecies.

Key words: ancient DNA, bighorn sheep, Cytochrome-*b* gene, displacement loop, extirpation, *Ovis canadensis*

Introduction

Bighorn sheep (*Ovis canadensis*) historically occurred in 16 mountain ranges (Davis and Taylor 1939) across the Trans-Pecos ecoregion of Texas (Gould 1962). During the 1930s and 1940s, W. B. Carson discovered pictographs of bighorn sheep in a cave in Victorio Canyon of the Sierra Diablos (Jackson 1938).

Although the dating was uncertain, it established a presence of bighorn sheep in the Trans-Pecos ecoregion before European occupation. Further, petroglyphs, which are known to predate those pictographs by several millennia, featuring bighorn sheep have been recorded in El Paso, Hudspeth, and Culberson counties (Buechner 1960). Archaeological evidence recovered from several caves in Texas (Eagle, Lower Sloth, and Williams Caves) indicated an even earlier occupation of bighorn sheep in the Trans-Pecos ecoregion; perhaps as early as 15,000–10,000 years before present (Ayer 1936; Geist 1985; Mead et al. 2021). Little evidence exists regarding population sizes, although Cook (1994) reviewed early reports of Texas Parks and Wildlife personnel, biologists, and eye-witness accounts of ranchers and landowners and estimated a population size of 1,500 individuals in the 1880s. During the biological survey of Texas, Vernon Bailey (Bailey 1905) occasionally encountered bighorn sheep and estimated that approximately 500 individuals inhabited the Trans-Pecos ecoregion and southeastern New Mexico during the early 1900s. Eventually, conservation efforts were established, among which was a ban on hunting in 1903. However, this was lightly enforced, if at all. Predator control efforts from 1929 to 1939 (reduction of mountain lions, *Puma concolor* and golden eagles, *Aquila chrysaetos*) had varying levels of success; Davis and Taylor (1939) reported the population size of bighorn sheep did not increase, whereas Carson (1941) stated it did. Davis and Taylor (1939) and Carson (1941) attributed competition for food and water resources, coupled with disease transmission from domestic livestock, as effectively eliminating bighorn sheep from mountain ranges. Regardless of the reason(s) for population reduction, only 14 bighorn sheep remained in the Sierra Diablos by 1959 (Cook 1994) and by 1960, two individuals remained in Victorio Canyon (Kilpatric 1982). Though the exact date remains uncertain, it appears extirpation of Texas bighorn sheep occurred during the early 1960s.

During Bailey's biological survey (Bailey 1905), he collected six bighorn sheep specimens (i.e., complete skulls, or skins, or both) from the Guadalupe and Van Horn Mountains in 1901 and 1902, respectively. Based on phenotypic characteristics from the skulls of four males (4–7 years old), he assigned the Texas populations of bighorn sheep to the formerly recognized subspecies *O. c. mexicana.* In 1912, Bailey reexamined the skulls of the same males and two previously unexamined females and discovered that unique cranial characteristics were prominent in only the females. Further, Bailey (1912) reported that bighorn sheep in Texas and southeastern New Mexico were exceptionally different from previously described subspecies (*O. c. mexicana*, *O. c. auduboni*, and *O. c. canadensis*) based on "extremely narrow" facial regions of the skull, nasals, and palate. This identification of different cranial characteristics led Bailey to describe bighorn sheep in Texas and southeastern New Mexico as a distinct taxonomic unit, *O. c. texianus* (Bailey 1912). This subspecific designation was later reviewed by Cowan (1940), who acknowledged the cranial differences as suggested by Bailey (1912) but demonstrated that these measurements were not statistically inimitable from specimens of *O. c. mexicana* from Chihuahua. Consequently, Cowan (1940) synonymized *O. c. texianus* with *O. c. mexicana*, which was recognized by Miller and Kellogg (1955) of the United States National Museum and remains as such today.

In the desert southwest of North America, there exist four putative subspecies of desert bighorn sheep (DBS): 1) *O. c. cremnobates* (Elliot 1903); 2) *O. c. mexicana* (Merriam 1901); 3) *O. c. nelsoni* (Merriam 1897); and 4) *O. c. weemsi* (Goldman 1937). Early mitochondrial DNA (mtDNA) and morphologic studies (Wehausen and Ramey 1993; Ramey 1995) suggested synonymizing all four DBS subspecies and retaining *O. c. nelsoni* (taxonomic priority) as a single polytypic subspecies. Several later studies (Boyce et al. 1999; Epps et al. 2010; Buchalski et al. 2016; Wright 2023) have identified several haplotypes among *O. c. cremnobates*, *O. c. mexicana*, and *O. c. nelsoni*; however, the genetic divergence among these haplotypes were not supported by phylogenetic analyses, which remains in agreement with Ramey (1995). However, microsatellite (Buchalski et al. 2016; Gille et al. 2019; Creech et al. 2020) and genomic data (Wright et al. 2024) indicated genomic distinctions between three DBS subspecies (*O. c. cremnobates*, *O. c. mexicana*, and *O. c. nelsoni*) to the exclusion of *O. c. weemsi* (no genetic samples available). Based on these prior genetic and morphological results and for ease of reference, we retain the use of putative subspecific designations as follows: *O. c. cremnobates* associated with the Peninsular Ranges (Sonoran Desert); *O. c. mexicana* associated with the Sonoran Desert; *O. c. nelsoni* associated

with the Great Basin and Mojave Deserts; and *O. c. texianus* associated with the Chihuahuan Desert in the Trans-Pecos Region of Texas.

Given the restricted geographic distribution of *O. c. texianus* and its ultimate extinction (Fig. 1), it is unknown whether it represented a valid taxon or if the cranial characters examined by Bailey (1912) and Cowan (1940) were products of age, sex, or other forms of morphological variation. Therefore, the objective of this study was to examine genetic variation in known pre-extirpation individuals (*O. c. texianus*) compared to contemporary populations of DBS to determine if *O. c. texianus* was indeed a unique taxon. Because *O. c. texianus* was extirpated by the 1960s, reconstructing its genetic affiliation required obtaining bone fragments, dried muscle, skin clips, horn shavings, or hair samples from museum specimens and trophy mounts. MtDNA markers (Cytochrome-*b*, *Cyt*b; displacement loop, D-loop) were used to: 1) generate a haplotype profile for *O. c. texianus*; 2) examine the degree of variation in *O. c. texianus* compared to present-day DBS populations; and 3) determine if *O. c. texianus* was a member of desert or Rocky Mountain bighorn sheep.

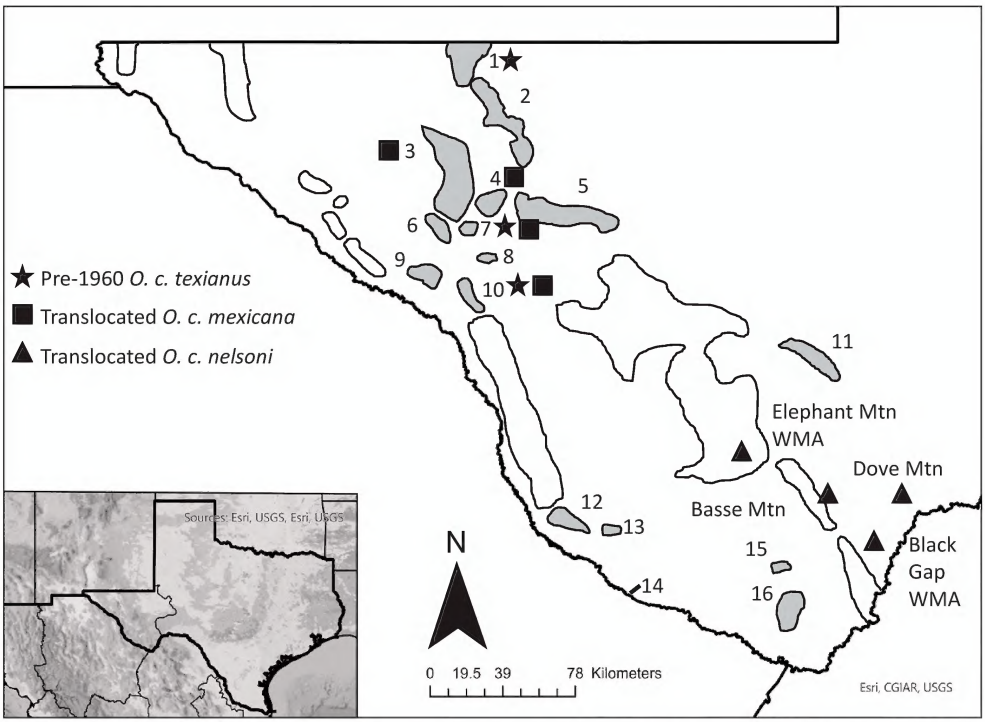

Figure 1. Map depicting sites for genetic sampling: stars represent pre-1960 individuals of presumed *O. c. texianus*; squares represent descendants of previously translocated *O. c. mexicana*; and triangles squares represent descendants of previously translocated *O. c. nelsoni*. Shaded areas represent historic distributions of bighorn sheep in the Trans-Pecos region based on Davis and Taylor (1939). Wildlife Management Areas and mountains were abbreviated to WMA and Mtn, respectively. Numbers represent: 1, Guadalupe Mountains; 2, Delaware Mountains; 3, Sierra Diablo; 4, Baylor Mountains; 5, Apache Mountains; 6, Carrizo Mountains; 7, Beach Mountains; 8, Wylie Mountains; 9, Eagle Mountains; 10, Van Horn Mountains; 11, Glass Mountains; 12, Chinati Mountains; 13, Cienega Mountains; 14, Grand Canyon of the Rio Grande; 15, Corozones Mountains; and 16, Chisos Mountains.

MATERIALS AND METHODS

Sampling.—Individuals (*Cyt*b: n = 8; D-loop: n = 21) representative of *O. c. texianus* (historic population, collected 1901–1940s) were obtained through three sources: 1) natural history collections (National Museum of Natural History, USNM; Guadalupe Mountains National Park, GUMO; Earnest and Dorothy Barrow Foundation Museum); 2) private collections of Texas Bighorn Society members; and 3) opportunistic skeletal retrieval on private ranches. Sequences were generated and obtained from GenBank to increase the sampling size and geographic distribution of contemporary populations. It is important to note subspecies that shared similar haplotypes based on mtDNA data from GenBank are noted as such by including both subspecies (separated by a slash) for that accession number. These include *O. c. californiana* (*Cyt*b: n = 1; D-loop: n = 4), *O. c. canadensis* (*Cyt*b: n = 18; D-loop: n = 57), *O. c. californiana/canadensis* (*Cyt*b: n = 0; D-loop: n = 1), and *O. c. sierrae* (*Cyt*b: n = 0; D-loop: n = 1), as well as representatives of DBS, including *O. c. cremnobates* (*Cyt*b: n = 1; D-loop: n = 17), *O. c. mexicana* (*Cyt*b: n = 8; D-loop: n = 45), *O. c. nelsoni* (*Cyt*b: n = 13; D-loop: n = 57), *O. c. californiana/nelsoni* (*Cyt*b: n = 0; D-loop: n = 1), and *O. c. mexicana/nelsoni* (*Cyt*b: n = 0; D-loop: n = 5). We also included one (*Cyt*b: n = 0; D-loop: n = 1) unpublished sequence (AY116623) for Weems bighorn sheep (*O. c. weemsi*) archived on the GenBank nucleotide database. See the Appendix for more detailed information.

DNA sequencing.—For newly collected samples, genomic DNA was isolated from 0.1g of liver, skeletal muscle, or ear clip tissue using the Qiagen DNeasy kit (Qiagen, Valencia, California). For the mitochondrial *Cyt*b gene, the full-length gene (1,143 bp) was amplified using polymerase chain reaction (PCR) methodology following Saiki et al. (1988) with the amplification primers LGL765 (forward, Bickham et al. 1995) and LGL766 (reverse, Bickham et al. 2004). HotStarTaq (Qiagen Inc., Valencia, California) was used in the PCR along with the following thermal profile: hot start of 80°C, initial denaturation at 95°C for 2 min, followed by 34 cycles of denaturation at 95°C for 30 s, annealing (range: 44–45°C) for 45 s, extension at 73°C for 1 min, and a final extension at 73°C for 15 min.

For the mtDNA displacement loop (D-loop), the full-length region (971 bp not yet aligned to outgroup taxa) was amplified as above with the following primers: 2340-4 (forward) and 2340-5 (reverse, Castro-Campillo et al. 1999). Thermal profiles for PCR were as follows: a hot start of 80°C, initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing (range: 47–48°C) for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 15 min.

All PCR products were purified with ExoSAP-IT (Applied Biosystems, Foster City, California). Cycle sequence primers for *Cyt*b were LGL765, LGL766, 870R, and F1 (Peppers et al. 2002; Whiting et al. 2003) and for D-Loop included 2340-4, 2340-5, and 1115 (Méndez-Harclerode et al. 2005). Subsequent cycle sequencing reactions were purified using the ABI Prism Big Dye version 3 terminator ready reaction mix (Applied Biosystems, Foster City, California). Sequencing reactions were purified using Sephadex columns (Princeton Separation, Adelphia, New Jersey) and centrifugation, followed by dehydration. Purified products were analyzed on an ABI Prism 310 automated sequencer (Eurofins Genomics LLC, Louisville, Kentucky).

Ancient DNA was isolated following a modification of methods reported in Curry and Derr (2019) and Wright et al. (2020). Bone fragments, horn shavings, skin clips, and dried muscle tissue samples initially were cleaned using a 95% ethanol rinse and then immediately treated with UV irradiation for 5 min. Samples were then pulverized using a mortar and pestle with liquid nitrogen. Each skin clip sample was washed with ddH_2O and incubated at 56°C for 15 minutes (3 repetitions). Genomic DNA was isolated using the Qiagen DNeasy kit (Qiagen, Valencia, California). The *Cyt*b and D-loop markers were amplified using PCR methods with primers (Table 1) designed to capture partial sequences of variable regions. Cytiva PureTaq Ready-To-Go PCR Beads (Cytiva Life Sciences, Marlborough, Massachusetts) were used following the recommended protocol of 20 µL ddH_2O, 4 µL DNA, and 0.5 µL of each primer. The same thermal profile was used as described above for contemporary samples

Table 1. Primers for mitochondrial markers (Cytochrome-*b* and displacement loop) obtained from ancient DNA samples of *Ovis canadensis texianus* and their associated approximate fragment size and location on each respective marker. Locations for Cytochrome-*b* and displacement loop were based on OQ692309 and OQ72508, respectively.

MtDNA marker	Primer Pair	Primer Name	Primer Sequence	Approximate Fragment Size (base pairs)	Location on the Gene (base pairs)
Cytochrome-*b*					
	1	dbs1_F	5'-ACA AAA TCC CCT TCC ACC CC-3'	323	Start: 647 End: 969
		dbs1_R	5'-GCA TTG GCT GAT AGG TCG GA-3'		
	2	dbs2_F	5'-TTC ATG CAT GTA GGA CGG GG-3'	384	Start: 283 End: 666
		dbs2_R	5'-GGG GTG GAA GGG GAT TTT GT-3'		
	3	dbs3_F	5'-CAC GCA AAC GGA GCA TCA AT-3'	335	Start: 247 End: 581
		dbs3_R	5'-ATG GCG AGG GCT GCA ATT AT-3'		
	4	dbs_4F	5'-ACA TCC GAA AAA CCC ACC CA-3'	257	Start: 8 End: 264
		dbs_4R	5'-TGA TGC TCC GTT TGC GTG TA-3'		
	5	dbs_5F	5'-CCC ACA TTT GCC GAG ACG TA-3'	346	Start: 200 End: 545
		dbs_5R	5'-TGG AAG GCA AAG AAT CGG GT-3'		
	6	dbs_6F	5'-TAA TTG CAG CCC TCG CCA TA-3'	365	Start: 563 End: 927
		dbs_6R	5'-TGT GTG GAG GAG GGG CAT AA-3'		
	7	dbs_7F	5'-AGT CCT CGC CCT AAT CCT CT-3'	156	Start: 870 End: 1025
		dbs_7R	5'-GGC TGG CCT CCA ATT CAT GT-3'		
Displacement loop					
	1	BHS_DLOOP1F	5'-ATC TAC CAT GCC GCG TGA AA-3'	276	Start: 570 End: 845
		BHS_DLOOP1R	5'-TTG ACG GCC ATA GCT GAG TC-3'		
	2	BHS_DLOOP2F	5'-AAA AGC ACA CCA TCC ACC CA-3'	355	Start: 235 End: 589
		BHS_DLOOP2R	5'-TTT CAC GCG GCA TGG TAG AT-3'		
	3	BHS_DLOOP3F	5'-AGG AGA ACA ACC AAC CTC CC-3'	200	Start: 1 End: 200
		BHS_DLOOP3R	5'-GTG GAG TGG AAA GTC CGT GT-3'		
	4	BHS_DLOOP4F	5'-TCA GCT ATG GCC GTC AAA GG-3'	287	Start: 829 End: 1115
		BHS_DLOOP4R	5'-ATG TAT GAG ACC CAG GTG CC-3'		

with the exception that the annealing temperature was 52°C and 51–52°C for *Cyt*b and D-loop, respectively. Primers used to cycle sequence the products included those listed in Table 1.

Resulting sequences were aligned and proofed with Sequencher 4.10.1 software (Gene Codes Corporation, Ann Arbor, Michigan) and chromatograms were inspected to authenticate any base changes. All DNA sequences obtained in this study were deposited in NCBI GenBank (*Cyt*b: OQ692293–OQ692336; D-loop: OQ725063–OQ725142).

Phylogenetic analyses.—For the *Cyt*b dataset, 66 individuals (42 sampled herein and 24 acquired from NCBI GenBank) were included for analyses (see Appendix). The domestic sheep (*Ovis aries*) was designated as the outgroup species with ingroup individuals of Dall sheep (*O. dalli*) and Snow sheep (*O. nivicola*) added as reference samples (see Appendix). A parsimony analysis (PAUP* Version 4.0a169, Swofford 2003) was conducted to identify synapomorphies indicative of taxonomic identifications. Parsimony characters were assigned equal weight and variable nucleotide positions were treated as unordered, discrete characters with four possible states: A, C, G, and T. Phylogenetically uninformative characters were removed from the analysis. The most parsimonious trees were estimated using the heuristic search and tree-bisection-reconnection option. A strict consensus tree was generated from the population of most-parsimonious trees and a subsequent bootstrap analysis (Felsenstein 1985) with 1,000 iterations and the "fast" step-wise option selected to evaluate nodal support.

Eighty-eight maximum likelihood (ML) models were evaluated using jModelTest-2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012). The Akaike information criterion with a correction for finite sample sizes (AICc, Hurvich and Tsai 1989; Burnham and Anderson 2004) identified the Hasegawa-Kishino-Yano model of nucleotide substitution (HKY, Hasegawa et al. 1985) and proportion of invariable sites model (HKY+I, -lnL = 2205.0851) as the most appropriate for the *Cyt*b dataset. However, the general time reversible (Tavaré 1986) plus proportion of invariable sites plus gamma distribution (GTR+I+Γ) model of nucleotide substitution, the most complex model, has been suggested to fit real data better than simpler models (Jayaswal et al. 2011; Sumner et al. 2012; Arenas 2015). We attempted several test runs using both models of HKY+I and GTR+I+Γ and determined that topologies and support did not differ. Therefore, we proceeded with the GTR+I+Γ model for all analyses. A likelihood analysis was performed using RAxML (Version 8.2.12, Stamatakis 2014) and the following parameters: base frequencies (A = 0.3135, C = 0.2897, G = 0.1261, and T = 0.2707), and the GTR+I+Γ. Nodal support was evaluated using the bootstrap method (1,000 iterations, Felsenstein 1985), with bootstrap values (BS) ≥ 65 used to indicate moderate-to-strong nodal support.

A ML analysis using a Bayesian inference (BI) model (MrBayes v3.2.6, Ronquist et al. 2012) was conducted to generate posterior probability values (PPV). The GTR+I+Γ nucleotide substitution model and the following parameters were used: two independent runs with four Markov-chains (one cold and three heated; MCMCMC), 10 million generations, and sample frequency of every 1,000 generations from the last nine million generated. A visual inspection of likelihood scores resulted in the first 1,000,000 trees being discarded (10% burn-in) and a consensus tree (50% majority rule) constructed from the remaining trees. PPV ≥ 0.95 were used to determine nodal support (Huelsenbeck et al. 2002).

The above phylogenetic methodologies similarly were applied to the D-loop datasets, which included domestic sheep (*O. aries*) designated as the outgroup and Dall (*O. dalli*) and Snow (*O. nivicola*) sheep as ingroup taxa (see Appendix). The differences in phylogenetic methods for D-loop are described below. Due to the lack of full-length sequences for all individuals (samples collected herein and sequences obtained from NCBI GenBank), two datasets were used to examine relationships using the D-loop marker: 1) a dataset of 235 individuals that included all 21 individuals representative of *O. c. texianus* (regardless of sequence length), and 2) a reduced dataset (n = 214) that only included two individuals that are representative of *O. c. texianus* (see Appendix). These latter two individuals were the only representatives of *O. c. texianus* that possessed complete sequences (1,290 bp when aligned to out- and ingroup taxa).

Eighty-eight ML models were evaluated using jModelTest-2.1.10 (Guindon and Gascuel 2003;

Darriba et al. 2012). The AICc (Hurvich and Tsai 1989; Burnham and Anderson 2004) identified the transversion model of nucleotide substitution, proportion of invariable sites, and gamma distribution (full dataset: TVM+I+Γ, -lnL = 5340.1427; reduced dataset: TVM+I+Γ, -lnL = 5002.4404) as the most appropriate for D-loop datasets.

For the full D-loop dataset, a likelihood analysis was performed using the following parameters: base frequencies (A = 0.3551, C = 0.2241, G = 0.1370, and T = 0.3839), proportion of invariable sites (I = 0.5040), and gamma distribution (G = 0.6700) and the GTR+I+Γ in the program RAxML (Version 8.2.12, Stamatakis 2014). For the reduced D-loop dataset, a likelihood analysis was performed using the following parameters: base frequencies (A = 0.3483, C = 0.2206, G = 0.1431, and T = 0.2881), proportion of invariable sites (I = 0.5430), and gamma distribution (G = 0.6600) and the GTR+I+Γ in the program RAxML (Version 8.2.12, Stamatakis 2014).

Genetic divergence.—Genetic distance values for selected taxa and mitochondrial haplogroups were estimated using the Kimura 2-parameter model of evolution (Kimura 1980) and the Tamura-Nei model of evolution (Tamura and Nei 1993) for the *Cyt*b and D-loop datasets, respectively, using the program MEGA 11 (Tamura et al. 2018). The resulting values calculated from the mitochondrial markers were used to examine levels of genetic divergence pertaining to the genetic species concept as outlined in Bradley and Baker (2001) and Baker and Bradley (2006).

RESULTS

Phylogenetic Analyses

Cytochrome-b *dataset*.—For the *Cyt*b dataset, the three phylogenetic analyses (BI, ML, and parsimony) generated similar topologies; therefore, only the topology obtained from the BI analysis is shown (Fig. 2). In the ML analysis, individuals representative of *O. dalli* were placed as sister taxa to all bighorn sheep. The BI and parsimony analyses placed *O. nivicola* and *O. dalli* as basal and collapsed all individuals of bighorn sheep into a single clade; consequently, only the BI and parsimony analyses are discussed in detail below. Although there was a lack of nodal support (bootstrap and posterior probability values), there were four nucleotide substitutions that were diagnostic between members of Groups 1 and 2 (Fig. 2).

In the BI analysis, two unsupported groupings were identified (I and II; Fig. 2). Group I contained 47 individuals from the US (Arizona, California, Colorado, New Mexico, and Texas) and Mexico (Coahuila) representing *O. c. nelsoni* and the formerly recognized desert subspecies *O. c. cremnobates*, *O. c. mexicana*, and *O. c. texianus*. Group II contained 19 individuals from the US (Idaho, New Mexico, and Washington) and Canada (Alberta).

Due to computation limitations for the parsimony analysis, the heuristic search was terminated before the analysis could be completed. At the point of termination (total number of rearrangements tried = 34,277,376), 905,332 equally, most-parsimonious trees (length = 118, homoplasy index = 0.0932, and consistency index = 0.9068) were retrieved. The parsimony analysis produced a topology (not shown) that essentially was identical to the topology of the BI analysis. There was no nodal support (BS < 65) for either Groups I or II resulting in an unresolved polytomy containing all represented subspecies of bighorn sheep excluding *O. c. sierrae* (no samples available). Four nucleotide substitutions determined to be phylogenetically informative (C69T, T669C, A843G, and A957T) and providing delineation between Groups I and II were superimposed onto the topology obtained from the BI analyses.

D-loop dataset.—As described above, two datasets were analyzed. In the first analysis, 21 representatives of *O. c. texianus* were included. For this dataset, the three phylogenetic analyses (BI, ML, and parsimony) showed low support (BS < 65, PPV < 0.95) for both Rocky Mountain and DBS clades (unresolved polytomy). In general, the 21 individuals of *O. c.*

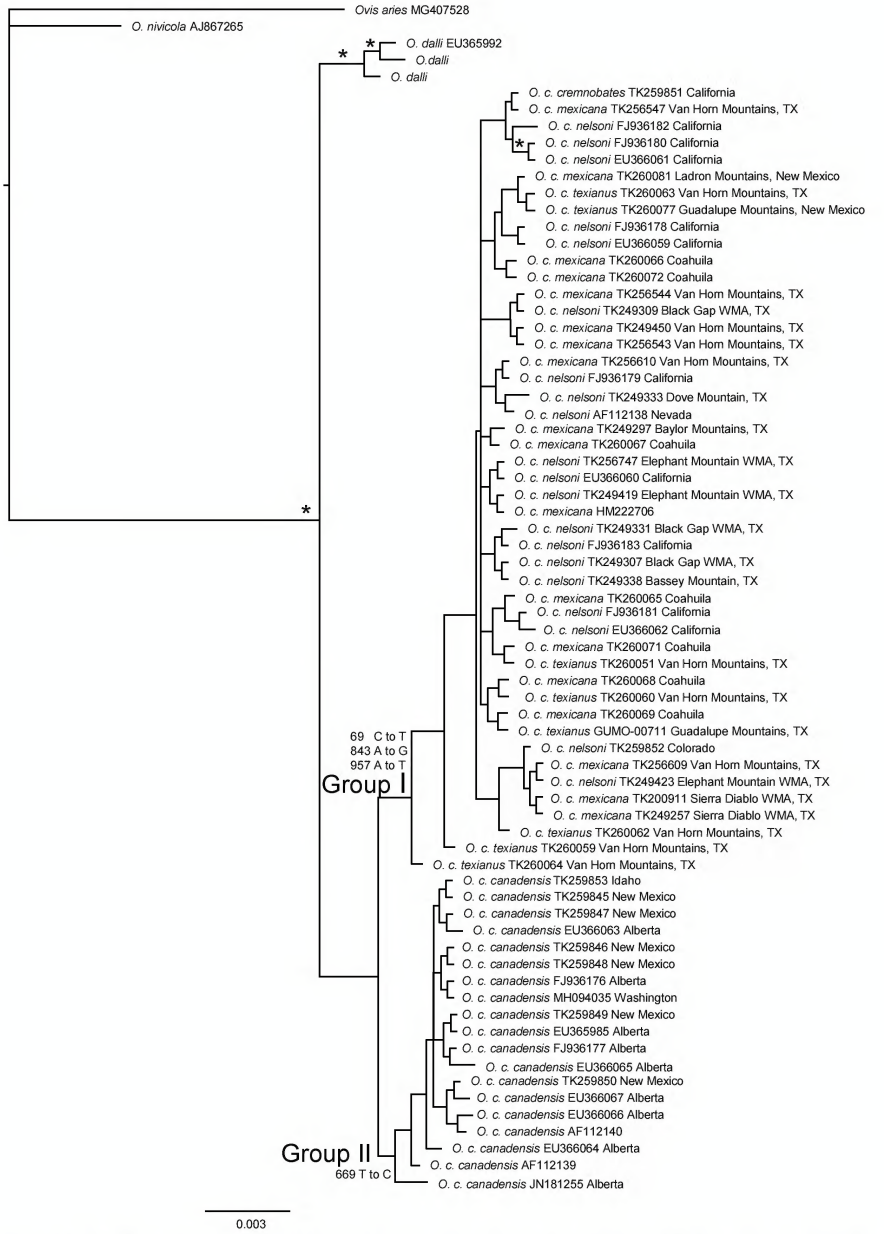
Ovis aries MG407528
O. nivicola AJ867265
O. dalli EU365992
O.dalli
O. dalli
O. c. cremnobates TK259851 California
O. c. mexicana TK256547 Van Horn Mountains, TX
O. c. nelsoni FJ936182 California
O. c. nelsoni FJ936180 California
O. c. nelsoni EU366061 California
O. c. mexicana TK260081 Ladron Mountains, New Mexico
O. c. texianus TK260063 Van Horn Mountains, TX
O. c. texianus TK260077 Guadalupe Mountains, New Mexico
O. c. nelsoni FJ936178 California
O. c. nelsoni EU366059 California
O. c. mexicana TK260066 Coahuila
O. c. mexicana TK260072 Coahuila
O. c. mexicana TK256544 Van Horn Mountains, TX
O. c. nelsoni TK249309 Black Gap WMA, TX
O. c. mexicana TK249450 Van Horn Mountains, TX
O. c. mexicana TK256543 Van Horn Mountains, TX
O. c. mexicana TK256610 Van Horn Mountains, TX
O. c. nelsoni FJ936179 California
O. c. nelsoni TK249333 Dove Mountain, TX
O. c. nelsoni AF112138 Nevada
O. c. mexicana TK249297 Baylor Mountains, TX
O. c. mexicana TK260067 Coahuila
O. c. nelsoni TK256747 Elephant Mountain WMA, TX
O. c. nelsoni EU366060 California
O. c. nelsoni TK249419 Elephant Mountain WMA, TX
O. c. mexicana HM222706
O. c. nelsoni TK249331 Black Gap WMA, TX
O. c. nelsoni FJ936183 California
O. c. nelsoni TK249307 Black Gap WMA, TX
O. c. nelsoni TK249338 Bassey Mountain, TX
O. c. mexicana TK260065 Coahuila
O. c. nelsoni FJ936181 California
O. c. nelsoni EU366062 California
O. c. mexicana TK260071 Coahuila
O. c. texianus TK260051 Van Horn Mountains, TX
O. c. mexicana TK260068 Coahuila
O. c. texianus TK260060 Van Horn Mountains, TX
O. c. mexicana TK260069 Coahuila
O. c. texianus GUMO-00711 Guadalupe Mountains, TX
69 C to T
843 A to G
957 A to T
Group I
O. c. nelsoni TK259852 Colorado
O. c. mexicana TK256609 Van Horn Mountains, TX
O. c. nelsoni TK249423 Elephant Mountain WMA, TX
O. c. mexicana TK200911 Sierra Diablo WMA, TX
O. c. mexicana TK249257 Sierra Diablo WMA, TX
O. c. texianus TK260062 Van Horn Mountains, TX
O. c. texianus TK260059 Van Horn Mountains, TX
O. c. texianus TK260064 Van Horn Mountains, TX
O. c. canadensis TK259853 Idaho
O. c. canadensis TK259845 New Mexico
O. c. canadensis TK259847 New Mexico
O. c. canadensis EU366063 Alberta
O. c. canadensis TK259846 New Mexico
O. c. canadensis TK259848 New Mexico
O. c. canadensis FJ936176 Alberta
O. c. canadensis MH094035 Washington
O. c. canadensis TK259849 New Mexico
O. c. canadensis EU365985 Alberta
O. c. canadensis FJ936177 Alberta
O. c. canadensis EU366065 Alberta
O. c. canadensis TK259850 New Mexico
O. c. canadensis EU366067 Alberta
O. c. canadensis EU366066 Alberta
O. c. canadensis AF112140
Group II
O. c. canadensis EU366064 Alberta
O. c. canadensis AF112139
669 T to C
O. c. canadensis JN181255 Alberta
0.003

Figure 2. Phylogeny of the Cytochrome-*b* gene using all individuals (n = 66). Bayesian posterior probability values are indicated by the * and represent ≥ 0.95 nodal support. Nucleotide substitutions that were phylogenetically informative (C69T, T669C, A843G, and A957T) and provided delineation between Groups I and II were superimposed onto the topology.

texianus were dispersed throughout the three topologies (not shown) and were associated with individuals of DBS and Rocky Mountain bighorn sheep. This was most likely caused by the lack of complete sequences across all samples. Therefore, all historic *O. c. texianus* samples were removed for the reduced dataset except for two individuals that possessed the sequence data for the entirety of the D-loop marker and subsequently reanalyzed.

For the reduced dataset, two clades were identified (I and II) in the BI analyses (Fig. 3). Clade I was supported and contained 150 individuals from the US (Arizona, California, Colorado, Nevada, New Mexico, Texas, and Wyoming) and Mexico (Baja California Sur and Coahuila). This clade primarily consisted of DBS representing the subspecies *O. c. nelsoni*, the formerly recognized subspecies *O. c. cremnobates*, *O. c. mexicana*, *O. c. texianus*, and *O. c. weemsi*, as well as contemporary populations of Texas bighorn sheep. In addition, Clade I contained a single *O. c. canadensis* sequence from Wyoming and a single haplotype representing individuals from Oregon (formerly recognized *O. c. californiana*) and Nevada (*O. c. nelsoni*). Clade II was unsupported but was subdivided into two supported subclades (A and B). Subclade-A contained two individuals from the US (California), including the Sierra Nevada subspecies (*O. c. sierrae*) and a single DBS sequence. Subclade II-B contained 62 individuals from the US (Arizona, Idaho, New Mexico, Oregon, Texas, Washington, and Wyoming) and Canada (Alberta and British Columbia). This subclade primarily consisted

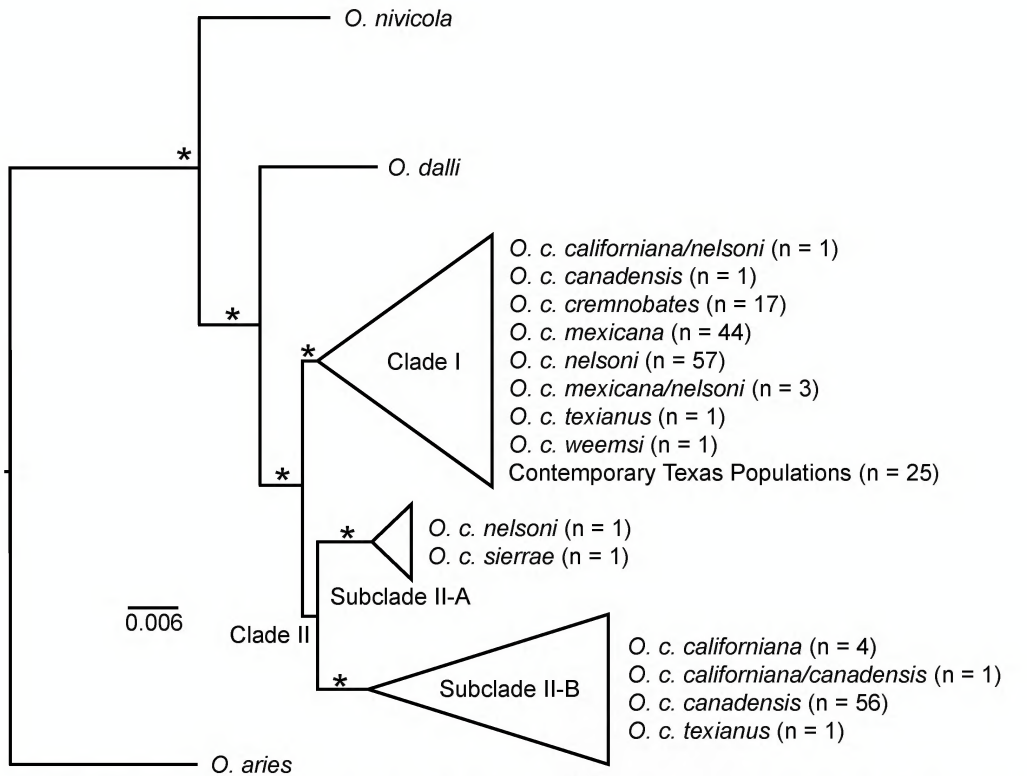
O. nivicola
O. dalli
O. c. californiana/nelsoni (n = 1)
O. c. canadensis (n = 1)
O. c. cremnobates (n = 17)
O. c. mexicana (n = 44)
O. c. nelsoni (n = 57)
O. c. mexicana/nelsoni (n = 3)
O. c. texianus (n = 1)
O. c. weemsi (n = 1)
Contemporary Texas Populations (n = 25)
Clade I
O. c. nelsoni (n = 1)
O. c. sierrae (n = 1)
Subclade II-A
0.006
Clade II
O. c. californiana (n = 4)
O. c. californiana/canadensis (n = 1)
O. c. canadensis (n = 56)
O. c. texianus (n = 1)
Subclade II-B
O. aries

Figure 3. Phylogeny of D-loop using selected individuals (n = 214). Bayesian posterior probability values are indicated by the * and represent ≥ 0.95 nodal support.

of Rocky Mountain bighorn sheep (*O. c. canadensis*), but also included California bighorn sheep (*O. c. californiana*) and one historic *O. c. texianus* sample.

Due to computation limitations for the parsimony analysis, the heuristic search was terminated before the analysis could be completed. At the point of termination (total number of rearrangements tried = 89,861,762), 917,993 equally, most-parsimonious trees (length = 1,001, homoplasy index = 0.7033, and consistency index = 0.1659) were retrieved. Twenty-nine nucleotide substitutions delineated Rocky Mountain bighorn sheep (Subclade II-B) from DBS (Clade I) whereas 5 nucleotide substitutions differed between Sierra Nevada and Nelson's bighorn sheep (Subclade II-A) and the remaining DBS sequences (Clade I).

Insertion Events

An insertion of a 75-base pair region was detected in some individuals of *O. c. canadensis* and *O. c. nelsoni* as well as the reference mitochondrial genome of *O. aries*. Several experimental analyses including and excluding this region did not result in a significantly different topology and therefore, the insertion was retained for all analyses.

Genetic Distances

Estimation of Kimura-2 parameter (Kimura 1980) genetic distances (Table 2), obtained from the *Cyt*b dataset, indicated that the average genetic distance among all Caprine individuals included in the study was 0.72%; whereas distances within selected taxa were as follows: 0.29% for individuals representing *O. dalli* and 0.35% for individuals representing bighorn sheep (Group I, DBS: 0.14%; Group II, Rocky Mountain bighorn sheep: 0.11%). Estimates for genetic distances between taxa were: 6.98% between *O. aries* and DBS; 4.90% between *O. nivicola* and DBS; 1.18 % between *O. dalli* and DBS; 6.77% between *O. aries* and Rocky Mountain bighorn sheep; 4.51% between *O. nivicola* and Rocky Mountain bighorn sheep; 1.11% between *O. dalli* and Rocky Mountain bighorn sheep; and 0.63% between DBS and Rocky Mountain bighorn sheep (Table 2).

Estimation of genetic distances (Table 3) using the Tamura and Nei (1993) model of evolution, obtained from the D-loop dataset, indicated that the average genetic distance among all individuals included in the study was 2.96%, whereas distances within individuals representing *O. canadensis* was 2.79%, and distances within selected taxa were as follows: Clade I, 1.67%; Subclade II-A, 1.59%; and Subclade II-B, 2.02%. Estimates for genetic distances between taxa were: 6.98% between Clade I and Subclade II-A; 4.90% between Clade I and Subclade II-B; and 1.18 % between Subclade II-A and Subclade II-B.

Table 2. Average genetic distances of mitochondrial Cytochrome-*b* sequences estimated using the Kimura 2-parameter model of evolution (Kimura 1980) for selected comparisons of bighorn sheep and taxa of Subfamily Caprinae. Groups I and II are in reference to Figure 1.

Comparison	Average Genetic Distance (%)
All Groups Overall Mean	0.72
Between	
Ovis aries vs *O. nivicola*	4.13
O. aries vs *O. dalli*	6.38
O. aries vs *O. canadensis*	6.92
O. nivicola vs *O. dalli*	4.30
O. nivicola vs *O. canadensis*	4.79
O. dalli vs *O. canadensis*	1.16
Within	
O. aries	NA
O. nivicola	NA
O. dalli	0.29
O. canadensis	0.35
Between	
O. aries vs Group I	6.98
O. nivicola vs Group I	4.90
O. dalli vs Group I	1.18
O. aries vs Group II	6.77
O. nivicola vs Group II	4.51
O. dalli vs Group II	1.11
Group I vs Group II	0.63
Within	
Group I	0.14
Group II	0.11

Table 3. Average genetic distances of mitochondrial D-loop sequences estimated using the Tamura-Nei model of evolution (Tamura and Nei 1993) for selected comparisons of bighorn sheep and taxa of the Subfamily Caprinae. Clade I and Subclades II-A and II-B are in reference to Figure 2.

Taxa	Genetic Distance (%)
All Groups Overall Mean	2.96
Between	
Ovis aries vs *O. nivicola*	10.00
O. aries vs *O. dalli*	10.50
O. aries vs *O. canadensis*	15.10
O. nivicola vs *O. dalli*	5.55
O. nivicola vs *O. canadensis*	6.34
O. dalli vs *O. canadensis*	5.78
Within	
O. aries	NA
O. nivicola	NA
O. dalli	NA
O. canadensis	2.79
Between	
O. aries vs Clade I	14.29
O. nivicola vs Clade I	5.88
O. dalli vs Clade I	5.46
O. aries vs Subclade II-A	16.21
O. nivicola vs Subclade II-A	6.94
O. dalli vs Subclade II-A	6.25
O. aries vs Subclade II-B	17.03
O. nivicola vs Subclade II-B	7.43
O. dalli vs Subclade II-B	6.52
Clade I vs Subclade II-A	3.92
Clade I vs Subclade II-B	4.23
Subclade II-A vs Subclade II-B	4.72
Within	
Clade I	1.67
Subclade II-A	1.59
Subclade II-B	2.02

DISCUSSION

DNA sequence variation in the mtDNA genome (*Cyt*b: n = 8; D-loop: n = 21) was examined for individuals representative of the historic distribution of *O. c. texianus* in Texas. The eight samples examined in the *Cyt*b dataset were comprised of individuals collected by W. B. Carson in the 1930s and 1940s in the Baylor, Beach, Diablo, and Van Horn mountains of Texas and a cave specimen found in the Guadalupe Mountains of New Mexico. From these analyses, all *O. c. texianus* individuals were genetically affiliated with DBS samples (Clade I, Fig. 2). Out of the 21 individuals used for the D-loop dataset, 19 were genetically similar to DBS and two individuals (obtained during the biological survey of Texas) nested within the Rocky Mountain bighorn sheep group. However, of the 21 individuals, only two individuals (one genetically similar to DBS, the other similar to Rocky Mountain bighorn sheep) representative of *O. c. texianus* possessed the full length sequence of D-loop. Therefore, in analyses using the reduced D-loop dataset, these two individuals were the only representatives of *O. c. texianus*. USNM118256 was placed in Subclade II-B (Fig. 3), which was comprised primarily of individuals of *O. c. canadensis*. This individual was collected south of Guadalupe Peak in the Guadalupe Mountains by Vernon Bailey in 1902. The other individual, TK260056, was collected in the area surrounding Van Horn, Texas (Baylor, Beach, Diablo, or Van Horn mountains) by W. B. Carson in the 1930s or 1940s and grouped with DBS (Clade I, Fig. 3). Although this latter outcome was to be expected, the subspecific identification of USNM118256 was not expected. Several postulates may explain this outcome: 1) the presence of the *O. c. canadensis* haplotype in populations surrounding the Guadalupe Mountains region; Ayer (1936) documented bone fragments belonging to both *O. c. auduboni*, which was synonymized with *O. c. canadensis* by Wehausen and Ramey (2000), and *O. c. texianus* in that region; 2) a specimen or museum tag or other identification measures were accidentally mishandled or incorrectly relabeled; 3) the vagility of bighorn sheep, in which long distance movements do occur and potentially allowed for the occasional range extension of Rocky Mountain bighorn into areas typical of DBS; 4) some level of hybridization occurred along the fringes of *O. c. canadensis* and DBS distributions that allowed for the capture of the *O. c. canadensis* haplotype into DBS; or 5) insufficient time has passed since the divergence of the subspecies for complete mtDNA lineage sorting. Unfortunately, only sequence data from D-loop were available for USNM118256; both *Cyt*b and D-loop are mtDNA markers and it may be presumed that the phylogenetic assignment using *Cyt*b would match that of the D-loop.

Further, one sequence (MK381324, WY7) representative of several *O. c. canadensis* from either Absaroka, Jackson, or Targhee populations in Wyoming (Love Stowell et al. 2020) was phylogenetically assigned to Clade I (Fig. 3) and was more similar to DBS. All other D-loop sequences representing unique haplotypes of *O. c. canadensis* in Wyoming grouped with the supported Subclade II-B (Fig. 3). Love Stowell et al. (2020) did not comment on this unique haplotype because their study was limited to *O. c. canadensis* and therefore did not include D-loop sequences of DBS. However, WY7 (MK381324) is basal to all other Rocky Mountain bighorn sheep, represented by a long branch length in the maximum likelihood phylogeny in Love Stowell et al. (2020) and is nested within Clade I herein (Fig. 3). This outcome suggests the presence of the desert subspecies of bighorn sheep in Wyoming populations, which is not expected considering the translocation history of the state (Wild Sheep Working Group 2015). The postulates regarding the *O. c. canadensis* haplotype in the Guadalupe Mountains (see above), including incomplete lineage sorting, may also provide relevant speculations in this scenario.

Although nodal support was low in the complete *Cyt*b and D-loop datasets, several diagnostic nucleotide substitutions delineate between the Rocky Mountain and DBS subspecies (Fig. 2) and may be an indication of recent genetic divergence among subspecies of bighorn sheep or a result of human-mediated translocations of bighorn sheep for the last 60 years. In addition, the polygynous reproductive strategy (one male with many females) of bighorn sheep may have given rise to several mitochondrial lineages in small populations. Although only one genetic study has been conducted on DBS in Texas (Wright et al. 2024), the individuals used in reintroductions were sourced from several regions where previous research has identified genetic differentiation among DBS populations at multiple geographic scales. Specifically, Buchalski et al. (2016)

used D-loop sequences to demonstrate that the three formerly recognized DBS subspecies (*O. c. cremnobates*, *O. c. mexicana*, and *O. c. nelsoni*) were distinct genetic populations (F_{ST} values ranged from 0.11–0.57, nucleotide diversity ranged from 0.0073–0.0128), although mitochondrial lineages could not be resolved when using phylogenetic analyses. Further, Gille et al. (2019) detected 30 unique mitochondrial haplotypes (pairwise F_{ST} values ranged from -0.15–0.97) using D-loop sequences among populations of both *O. c. nelsoni* and *O. c. mexicana* across Arizona (Gille et al. 2019). Herein, phylogenetic and genetic distance analyses indicated that contemporary populations of bighorn sheep in Texas are genetically similar to individuals representative of DBS.

Contemporary studies incorporating morphological, genetic, and genomic techniques have begun to clarify the subspecific designations of bighorn sheep. For example, Wehausen and Ramey (2000) examined cranial morphometrics of both extant and extinct bighorn sheep and synonymized *O. c. auduboni* (an extinct lineage) with *O. c. canadensis* (see Wehausen and Ramey 2000 for further distinctions). Wehausen and Ramey 1993 (morphological assessment), Ramey 1995 (mitochondrial DNA analysis), and Jessup and Ramey 1995 (allozyme data) found low genetic differentiation among the four formerly recognized DBS subspecies (*O. c. cremnobates*, *O. c. mexicana*, *O. c. nelsoni*, and *O. c. weemsi*) originally designated by Cowan (1940) and relegated them to a single polytypic subspecies, *O. c. nelsoni*. In contrast, subsequent studies using Major Histocompatibility Complex (MHC) and microsatellite loci (Boyce et al. 1997; Gutiérrez-Espeleta et al. 2000) revealed high levels of genetic variation among and within populations of the former DBS subspecies. Further, Gutiérrez-Espeleta et al. (2000) compared heterozygosity and genetic variation of DBS, excluding *O. c. weemsi*, to domestic sheep and determined bighorn populations were not depauperate in genetic variation as has been suggested. The Red Rock Refuge, New Mexico population (*O. c. mexicana*) had the lowest number of alleles ($N_A = 2.4$) and gene diversity ($H_E = 0.36$) whereas the Arizona populations (*O. c. mexicana* and *O. c. nelsoni*) were found to have some of the greatest levels of alleles (9–28) and gene diversity (0.44–0.60) for DBS comparable to Rocky Mountain bighorn sheep populations located near Wheeler Peak, New Mexico ($N_A = 3.2$, $H_E = 0.55$) and Sheep River, Alberta ($N_A = 4.4$, $H_E = 0.59$). Additionally, the rate of genetic differentiation as a function of geographic distance is much steeper among DBS than Rocky Mountain bighorn sheep, which ultimately may be due to larger population sizes or higher rates of gene flow among populations of Rocky Mountain bighorn sheep (Gutiérrez-Espeleta et al. 2000). Although these studies (Boyce et al. 1997; Gutiérrez-Espeleta et al. 2000) provide evidence of genetic differentiation within populations of DBS subspecies, the lack of phylogenetic support among populations led both studies to support Ramey's (1995) hypothesis that all DBS should be placed as a polytypic subspecies under *O. c. nelsoni*.

Recently, Buchalski et al. (2016) revisited the subspecific designations among three of the formerly recognized DBS subspecies (*O. c. cremnobates*, *O. c. mexicana*, and *O. c. nelsoni*) and included *O. c. sierrae* and *O. c. canadensis*, using microsatellite loci and D-loop sequences. Based on microsatellite data, all five groups formed unique genetic populations, where both genotypes of Sierra Nevada and Rocky Mountain bighorn sheep clustered separately along discriminant analysis of principal components (DAPC) axes. The three DBS subspecies clustered closely but remained independent of each other. Further, although Sierra Nevada and Rocky Mountain bighorn sheep formed well-supported clades, the three DBS subspecies represented a basal, poorly-supported clade based on a maximum likelihood phylogenetic analysis. Despite the polyphyletic relationship of the three DBS subspecies, which may be a result of incomplete lineage sorting or secondary contact relative to Sierra Nevada and Rocky Mountain bighorn sheep, the variation of mtDNA among Peninsular and Mexican bighorn sheep was divergent from the Nelson's subspecies. Overall, Buchalski et al. (2016) determined that DBS subspecies proposed by Cowan (1940), including *O. c. cremnobates*, *O. c. mexicana*, and *O. c. nelsoni*, represent distinct genetic populations that should be managed separately to maintain maximal biodiversity on the landscape. Other genomic datasets (microsatellites: Gille et al. 2019, Creech et al. 2020; RAD-seq: Wright 2023, Wright et al. 2024) were in agreement with the assessment of Buchalski et al. (2016), indicating genomic distinctions between three DBS subspecies

(*O. c. cremnobates*, *O. c. mexicana*, and *O. c. nelsoni*) to the exclusion of *O. c. weemsi* (no genetic samples available).

In conclusion, individuals of *O. c. texianus* sampled during the 1930s and 1940s in the Baylor, Beach, Diablo, and Van Horn mountains as well as contemporary populations of DBS in Texas most closely resemble mtDNA haplotypes of DBS and not *O. c. canadensis* (Rocky Mountain bighorn sheep). The conundrum of the genetic similarity of individuals of *O. c. canadensis* and one *O. c. texianus*, harvested in the Guadalupe Mountains in the early 1900s, warrants further investigation. Although phylogenetic support (Figs. 1 and 2) and genetic distances (Tables 2 and 3) were low and may not warrant genetic distinction (Bradley and Baker 2001; Baker and Bradley 2006) between *O. c. texianus* and the other DBS, morphological characters of the skull indicated sufficient differences that Bailey (1912) felt obligated to describe *O. c. texianus* as a distinct subspecies. Therefore, what is needed is a morphological study with an increased sample size as well as nuclear analyses (e.g., RAD-seq, whole genome sequencing, etc.) to identify the taxonomic lineage characteristic of *O. c. texianus*. Recent studies have used these methodologies (Jahner et al. 2019; Flesch et al. 2020); however, their focus was on regional populations of bighorn sheep, not range-wide. Finally, we recommend that curators and collection managers at natural history collections preserve and retain 'crusties' (i.e., dried muscle and skin) from historic specimens as dried muscle and skin clips outperformed bone and horn tissue samples for DNA extraction. Although most natural history collections clean skeletal remains using dermestid collections and by other means, it is paramount to allow dried tissue to remain on historic specimens that do not have tissue vouchers.

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Addresses of authors:

EMILY A. WRIGHT

Natural Science Research Laboratory
Museum of Texas Tech University
Lubbock, Texas 79409-3131 USA
emmyyy32@gmail.com

MICHAEL R. BUCHALSKI

Wildlife Genetics Research Unit
Wildlife Health Laboratory
California Department of Fish and Wildlife
Sacramento, California 95834 USA
Michael.buchalski@wildlife.ca.gov

ROBERT D. BRADLEY

Department of Biological Sciences and
Natural Science Research Laboratory
Museum of Texas Tech University
Lubbock, Texas 79409-3131 USA
robert.bradley@ttu.edu

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APPENDIX

List of individuals used in this study. The sample identification is provided by natural history collections (Earnest and Dorothy Barrow Foundation Museum; GUMO, Guadalupe Mountains National Park; TK, Natural Science Research Laboratory at the Museum of Texas Tech University; USNM, National Museum of Natural History, Smithsonian Institution) or genetic repositories (AZ, NV, PEBS, and YAQUI: California Department of Fish and Wildlife). NCBI GenBank Accession numbers are provided for both *Cyt*b and D-loop datasets. The subspecies classifications for Texas populations were based either on historic locality or Wright (2023). When possible, Sample IDs (no affiliation to natural history collections) are provided and are affiliated with publications that can be cross referenced by their GenBank accession numbers. Individuals that possessed sequences identical to those already on NCBI GenBank were not added to NCBI GenBank and are labeled as IDSG (identical sequences to GenBank).

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
Ovis aries	NA	MG407528	NA	Egypt
O. aries	NA	NA	NC001941	NA
O. nivicola	NA	AJ867265	NA	Russia: Taiganos Cape
O. nivicola	NA	NA	NC039431	Russia: Chersky Range
O. dalli	NA	EU365992	NA	Canada: Northwest Territories
O. dalli	TK259854	OQ692293	NA	USA: Alaska, Alaskan Range
O. dalli	TK259855	OQ692294	NA	USA: Alaska, Brooks Range
O. dalli	NA	NA	NC039432	USA: Alaska, Alaskan Range
O. canadensis canadensis	NA	FJ936176	NA	Canada: Alberta
O. c. canadensis	NA	FJ936177	NA	Canada: Alberta
O. c. canadensis	NA	EU365985	NA	Canada: Alberta
O. c. canadensis	NA	MH094035	NA	USA: Washington, Pullman
O. c. canadensis	NA	JN181255	NA	Canada: Alberta
O. c. canadensis	NA	EU366063	NA	Canada: Alberta
O. c. canadensis	NA	EU366064	NA	Canada: Alberta
O. c. canadensis	NA	EU366065	NA	Canada: Alberta
O. c. canadensis	NA	EU366066	NA	Canada: Alberta
O. c. canadensis	NA	EU366067	NA	Canada: Alberta
O. c. canadensis	NA	AF112139	NA	NA
O. c. canadensis	TK259845	OQ692299	OQ725064	USA: New Mexico, Taos Pueblo
O. c. canadensis	TK259846	OQ692300	OQ725065	USA: New Mexico, Taos Pueblo
O. c. canadensis	TK259847	OQ692301	OQ725066	USA: New Mexico, Taos Pueblo

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
O. c. canadensis	TK259848	OQ692302	OQ725067	USA: New Mexico, Taos Pueblo
O. c. canadensis	TK259849	OQ692303	OQ725068	USA: New Mexico, Taos Pueblo
O. c. canadensis	TK259850	OQ692304	OQ725069	USA: New Mexico, Taos Pueblo
O. c. canadensis	TK259853	OQ692298	OQ725063	USA: Idaho, Yellowjacket Mountain
O. c. canadensis	NA	NA	NC015889	Canada: Alberta, Ram Mountain
O. c. canadensis	NA	NA	MH094035	USA: Washington, Pullman
O. c. canadensis	NA	NA	AY091486	Canada: Rocky Mountains
O. c. canadensis	WY2	NA	MK381319	USA: Wyoming
O. c. canadensis	WY3	NA	MK381320	USA: Wyoming
O. c. canadensis	WY4	NA	MK381321	USA: Wyoming
O. c. canadensis	WY5	NA	MK381322	USA: Wyoming
O. c. canadensis	WY6	NA	MK381323	USA: Wyoming
O. c. canadensis	WY7	NA	MK381324	USA: Wyoming
O. c. canadensis	WY9	NA	MK381325	USA: Wyoming
O. c. canadensis	WY10	NA	MK381326	USA: Wyoming
O. c. canadensis	WY11	NA	MK381327	USA: Wyoming
O. c. canadensis	WY12	NA	MK381328	USA: Wyoming
O. c. canadensis	WY13	NA	MK381329	USA: Wyoming
O. c. canadensis	WY14	NA	MK381330	USA: Wyoming
O. c. canadensis	WY15	NA	MK381331	USA: Wyoming
O. c. canadensis	WY16	NA	MK381332	USA: Wyoming
O. c. canadensis	WY17	NA	MK381333	USA: Wyoming
O. c. canadensis	WY18	NA	MK381334	USA: Wyoming
O. c. canadensis	WY19	NA	MK381335	USA: Wyoming
O. c. canadensis	WY20	NA	MK381336	USA: Wyoming
O. c. canadensis	WY21	NA	MK381337	USA: Wyoming
O. c. canadensis	WY22	NA	MK381338	USA: Wyoming
O. c. canadensis	WY23	NA	MK381339	USA: Wyoming

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
O. c. canadensis	WY24	NA	MK381340	USA: Wyoming
O. c. canadensis	WY25	NA	MK381341	USA: Wyoming
O. c. canadensis	WY27	NA	MK381342	USA: Wyoming
O. c. canadensis	WY28	NA	MK381343	USA: Wyoming
O. c. canadensis	WY29	NA	MK381344	USA: Wyoming
O. c. canadensis	WY30	NA	MK381345	USA: Wyoming
O. c. canadensis	WY31	NA	MK381346	USA: Wyoming
O. c. canadensis	WY32	NA	MK381347	USA: Wyoming
O. c. canadensis	WY33	NA	MK381348	USA: Wyoming
O. c. canadensis	WY35	NA	MK381349	USA: Wyoming
O. c. canadensis	WY36	NA	MK381350	USA: Wyoming
O. c. canadensis	WY37	NA	MK381351	USA: Wyoming
O. c. canadensis	WY38	NA	MK381352	USA: Wyoming
O. c. canadensis	WY39	NA	MK381353	USA: Wyoming
O. c. canadensis	WY42	NA	MK381354	USA: Wyoming
O. c. canadensis	WY43	NA	MK381355	USA: Wyoming
O. c. canadensis	RM1	NA	KU363680	USA: New Mexico
O. c. canadensis	RM2	NA	KU363681	USA: New Mexico
O. c. canadensis	RM3	NA	KU363682	USA: New Mexico
O. c. canadensis	RM4	NA	KU363683	USA: New Mexico
O. c. canadensis	RM5	NA	KU363684	USA: New Mexico and Canada: British Columbia
O. c. canadensis	RM6	NA	KU363685	USA: New Mexico
O. c. canadensis	RM7	NA	KU363686	Canada: Alberta
O. c. canadensis	RM8	NA	KU363687	Canada: Alberta
O. c. canadensis	RM9	NA	KU363688	Canada: Alberta
O. c. canadensis	RM10	NA	KU363689	Canada: Alberta
O. c. californiana/nelsoni	A	NA	JX484768	USA: Oregon/USA: Nevada
O. c. californiana	B	NA	JX484769	USA: Oregon

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
O. c. californiana	C	NA	JX484770	USA: Oregon
O. c. californiana	D	NA	JX484771	USA: Oregon
O. c. californiana/canadensis	E	NA	JX484772	USA: Oregon/Canada: British Columbia
O. c. californiana	F	NA	JX484773	USA: Oregon
O. c. californiana	NA	AF112140	NA	NA
O. c. cremnobates	TK259851	OQ692295	OQ725084	USA: California, San Bernadino, 10 mi SE Barstow
O. c. cremnobates	PEBS_2020_044_019	NA	OQ725070	USA: California, Peninsular Ranges
O. c. cremnobates	PEBS_2020_004_027	NA	OQ725071	USA: California, Peninsular Ranges
O. c. cremnobates	PEBS_2020_004_032	NA	OQ725072	USA: California, Peninsular Ranges
O. c. cremnobates	PEBS_2020_004_051	NA	OQ725073	USA: California, Peninsular Ranges
O. c. cremnobates	3	NA	AF076911	USA: California, Peninsular Ranges
O. c. cremnobates	1	NA	AF076913	USA: California, Peninsular Ranges
O. c. cremnobates	7	NA	AF076914	USA: California, Peninsular Ranges
O. c. cremnobates	4	NA	AF076915	USA: California, Peninsular Ranges
O. c. cremnobates	6	NA	AF076916	USA: California, Peninsular Ranges
O. c. cremnobates	2	NA	AF076917	USA: California, Peninsular Ranges
O. c. cremnobates	PR1	NA	KU363660	Mexico: Peninsular Ranges
O. c. cremnobates	PR2	NA	KU363661	Mexico: Peninsular Ranges
O. c. cremnobates	PR3	NA	KU363662	Mexico: Peninsular Ranges
O. c. cremnobates	PR4	NA	KU363663	Mexico: Peninsular Ranges
O. c. cremnobates	H6	NA	KP688366	Mexico: Peninsular Ranges
O. c. cremnobates	H7	NA	KP688367	Mexico: Peninsular Ranges
O. c. mexicana	YAQUI1	NA	IDSG	Mexico: Sonora
O. c. mexicana	YAQUI2	NA	IDSG	Mexico: Sonora
O. c. mexicana	YAQUI3	NA	IDSG	Mexico: Sonora
O. c. mexicana	YAQUI4	NA	IDSG	Mexico: Sonora
O. c. mexicana	YAQUI5	NA	IDSG	Mexico: Sonora
O. c. mexicana	AZ95098	NA	OQ725074	USA: Arizona, Castle Dome Mountains

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
O. c. mexicana	AZ95099	NA	OQ725075	USA: Arizona, Castle Dome Mountains
O. c. mexicana	AZ95100	NA	OQ725076	USA: Arizona, Castle Dome Mountains
O. c. mexicana	AZ02009	NA	OQ725077	USA: Arizona, Kofa Mountains
O. c. mexicana	AZ02010	NA	OQ725078	USA: Arizona, Kofa Mountains
O. c. mexicana	AZ014431	NA	OQ725079	USA: Arizona, East Kofa Mountains
O. c. mexicana	AZ014434	NA	OQ725080	USA: Arizona, East Kofa Mountains
O. c. mexicana	TK260065	OQ692322	OQ725112	Mexico: Coahuila, Sierra del Carmen
O. c. mexicana	TK260066	OQ692323	OQ725113	Mexico: Coahuila, Sierra del Carmen
O. c. mexicana	TK260067	OQ692324	OQ725114	Mexico: Coahuila, Sierra del Carmen
O. c. mexicana	TK260068	OQ692325	OQ725115	Mexico: Coahuila, Sierra del Carmen
O. c. mexicana	TK260069	OQ692326	OQ725116	Mexico: Coahuila, Sierra del Carmen
O. c. mexicana	TK260070	NA	OQ725117	Mexico: Coahuila, Sierra del Carmen
O. c. mexicana	TK260071	OQ692327	OQ725118	Mexico: Coahuila, Sierra del Carmen
O. c. mexicana	TK260072	OQ692328	OQ725119	Mexico: Coahuila, Sierra del Carmen
O. c. mexicana	TK260081	OQ692297	OQ725086	USA: New Mexico, Ladron Mountains
O. c. mexicana	TK249297	OQ692313	NA	USA: Texas, Baylor Mountains
O. c. mexicana	TK249298	NA	OQ725098	USA: Texas, Baylor Mountains
O. c. mexicana	TK249301	NA	OQ725099	USA: Texas, Baylor Mountains
O. c. mexicana	TK249289	NA	OQ725100	USA: Texas, Beach Mountains
O. c. mexicana	TK249257	OQ692312	NA	USA: Texas, Sierra Diablo Wildlife Management Area
O. c. mexicana	TK249258	NA	OQ725094	USA: Texas, Sierra Diablo Wildlife Management Area
O. c. mexicana	TK249259	NA	OQ725095	USA: Texas, Sierra Diablo Wildlife Management Area
O. c. mexicana	TK249260	NA	OQ725096	USA: Texas, Sierra Diablo Wildlife Management Area
O. c. mexicana	TK249266	NA	OQ725097	USA: Texas, Sierra Diablo Wildlife Management Area
O. c. mexicana	TK200911	OQ692311	OQ725093	USA: Texas, Sierra Diablo Wildlife Management Area
O. c. mexicana	TK249450	OQ692305	OQ725087	USA: Texas, Van Horn Mountains
O. c. mexicana	TK256543	OQ692306	NA	USA: Texas, Van Horn Mountains
O. c. mexicana	TK256544	OQ692307	OQ725088	USA: Texas, Van Horn Mountains

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
O. c. mexicana	TK256547	OQ692308	NA	USA: Texas, Van Horn Mountains
O. c. mexicana	TK256548	NA	OQ725089	USA: Texas, Van Horn Mountains
O. c. mexicana	TK256575	NA	OQ725090	USA: Texas, Van Horn Mountains
O. c. mexicana	TK256609	OQ692309	OQ725091	USA: Texas, Van Horn Mountains
O. c. mexicana	TK256610	OQ692310	OQ725092	USA: Texas, Van Horn Mountains
O. c. mexicana	NA	HM222706	NA	NA
O. c. mexicana	T	NA	AY904013	USA: Arizona, Sonoran Desert
O. c. mexicana	U	NA	AY904014	USA: Arizona, Sonoran Desert
O. c. mexicana	W	NA	AY904016	USA: Arizona, Sonoran Desert
O. c. mexicana	CD1	NA	KU363638	USA: New Mexico, Chihuahuan Desert
O. c. mexicana	CD2	NA	KU363639	USA: New Mexico, Chihuahuan Desert
O. c. mexicana	SC1	NA	KU363664	USA: Arizona, Sonoran Desert/USA: New Mexico, Chihuahuan Desert
O. c. mexicana	SC2	NA	KU363665	USA: Arizona, Sonoran Desert/USA: New Mexico, Chihuahuan Desert
O. c. mexicana	SD1	NA	KU363666	USA: Arizona, Sonoran Desert
O. c. mexicana	SD2	NA	KU363667	USA: Arizona, Sonoran Desert
O. c. mexicana	SD3	NA	KU363668	USA: Arizona, Sonoran Desert
O. c. mexicana	SD4	NA	KU363669	USA: Arizona, Sonoran Desert
O. c. mexicana	SD6	NA	KU363670	USA: Arizona, Sonoran Desert
O. c. mexicana	SD7	NA	KU363671	USA: Arizona, Sonoran Desert
O. c. mexicana	SD8	NA	KU363672	USA: Arizona, Sonoran Desert
O. c. mexicana	SD9	NA	KU363673	USA: Arizona, Sonoran Desert
O. c. mexicana	SD10	NA	KU363674	USA: Arizona, Sonoran Desert
O. c. mexicana	SD11	NA	KU363675	USA: Arizona, Sonoran Desert
O. c. mexicana	SD12	NA	KU363676	USA: Arizona, Sonoran Desert
O. c. mexicana	SD13	NA	KU363677	USA: Arizona, Sonoran Desert
O. c. mexicana	SD14	NA	KU363678	USA: Arizona, Sonoran Desert
O. c. mexicana	SD15	NA	KU363679	USA: Arizona, Sonoran Desert
O. c. mexicana	Hap1	NA	AY116621	Mexico: Tiburon Island

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
O. c. mexicana	Hap2	NA	AY116622	Mexico: Tiburon Island
O. c. nelsoni	5	NA	AF076912	USA: California, Mojave Desert
O. c. nelsoni	NV98001	NA	IDSG	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV98003	NA	IDSG	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV98006	NA	IDSG	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV98007	NA	IDSG	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV98008	NA	IDSG	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV98009	NA	OQ725081	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV98010	NA	OQ725082	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV98011	NA	IDSG	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV98012	NA	IDSG	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV98017	NA	OQ725083	USA: Nevada, Gabbs Valley Range
O. c. nelsoni	NV94011	NA	IDSG	USA: Nevada, Muddy Mountains
O. c. nelsoni	NV94013	NA	IDSG	USA: Nevada, Muddy Mountains
O. c. nelsoni	NV94015	NA	IDSG	USA: Nevada, Muddy Mountains
O. c. nelsoni	NV94016	NA	IDSG	USA: Nevada, Muddy Mountains
O. c. nelsoni	NV94017	NA	IDSG	USA: Nevada, Muddy Mountains
O. c. nelsoni	NV94018	NA	IDSG	USA: Nevada, Muddy Mountains
O. c. nelsoni	NV94019	NA	IDSG	USA: Nevada, Muddy Mountains
O. c. nelsoni	NV94020	NA	IDSG	USA: Nevada, Muddy Mountains
O. c. nelsoni	TK259852	OQ692296	OQ725085	USA: Colorado, Gunnison River, Unit S62
O. c. nelsoni	TK249307	OQ692317	NA	USA: Texas, Black Gap Wildlife Management Area
O. c. nelsoni	TK249309	OQ692318	OQ725105	USA: Texas, Black Gap Wildlife Management Area
O. c. nelsoni	TK249331	OQ692319	NA	USA: Texas, Black Gap Wildlife Management Area, Reagan Canyon
O. c. nelsoni	TK249332	NA	OQ725106	USA: Texas, Black Gap Wildlife Management Area, Reagan Canyon
O. c. nelsoni	TK249338	OQ692320	NA	USA: Texas, Bassey Mountain
O. c. nelsoni	TK249339	NA	OQ725107	USA: Texas, Bassey Mountain
O. c. nelsoni	TK249340	NA	OQ725108	USA: Texas, Bassey Mountain

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
O. c. nelsoni	TK249333	OQ692321	NA	USA: Texas, Dove Mountain
O. c. nelsoni	TK249334	NA	OQ725109	USA: Texas, Dove Mountain
O. c. nelsoni	TK249337	NA	OQ725110	USA: Texas, Dove Mountain
O. c. nelsoni	TK249343	NA	OQ725111	USA: Texas, Dove Mountain
O. c. nelsoni	TK249344	NA	OQ725101	USA: Texas, Elephant Mountain Wildlife Management Area
O. c. nelsoni	TK249345	NA	OQ725102	USA: Texas, Elephant Mountain Wildlife Management Area
O. c. nelsoni	TK249416	NA	OQ725103	USA: Texas, Elephant Mountain Wildlife Management Area
O. c. nelsoni	TK249419	OQ692314	NA	USA: Texas, Elephant Mountain Wildlife Management Area
O. c. nelsoni	TK249423	OQ692315	NA	USA: Texas, Elephant Mountain Wildlife Management Area
O. c. nelsoni	TK256747	OQ692316	NA	USA: Texas, Elephant Mountain Wildlife Management Area
O. c. nelsoni	TK256759	NA	OQ725104	USA: Texas, Elephant Mountain Wildlife Management Area
O. c. nelsoni	NA	FJ936178	NA	USA: California
O. c. nelsoni	NA	FJ936179	NA	USA: California
O. c. nelsoni	NA	FJ936180	NA	USA: California
O. c. nelsoni	NA	FJ936181	NA	USA: California
O. c. nelsoni	NA	FJ936182	NA	USA: California
O. c. nelsoni	NA	FJ936183	NA	USA: California
O. c. nelsoni	NA	EU366059	NA	USA: California
O. c. nelsoni	NA	EU366060	NA	USA: California
O. c. nelsoni	NA	EU366061	NA	USA: California
O. c. nelsoni	NA	EU366062	NA	USA: California
O. c. nelsoni	NA	AF112138	NA	USA: Nevada, Smith Creek Cave
O. c. nelsoni	A	NA	AY903993	USA: California, Mojave Desert/USA: Arizona, Colorado Plateau
O. c. nelsoni	B	NA	AY903995	USA: California, Mojave Desert
O. c. nelsoni	C	NA	AY903996	USA: California, Mojave Desert
O. c. nelsoni	D	NA	AY903997	USA: California, Mojave Desert
O. c. nelsoni	E	NA	AY903998	USA: California, Mojave Desert/USA: Arizona, Colorado Plateau/USA: Utah, Colorado Plateau

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
O. c. nelsoni	F	NA	AY903999	USA: California, Mojave Desert
O. c. nelsoni	G	NA	AY904000	USA: California, Mojave Desert
O. c. nelsoni	H	NA	AY904001	USA: California
O. c. nelsoni	I	NA	AY904002	USA: California, Mojave Desert
O. c. nelsoni	J	NA	AY904003	USA: California
O. c. nelsoni	K	NA	AY904004	USA: California
O. c. nelsoni	L	NA	AY904005	USA: California
O. c. nelsoni	M	NA	AY904006	USA: Nevada, Colorado Plateau
O. c. nelsoni	N	NA	AY904007	USA: California, Mojave Desert and Transverse Ranges
O. c. nelsoni	O	NA	AY904008	USA: California
O. c. nelsoni	P	NA	AY904009	USA: California
O. c. nelsoni	Q	NA	AY904010	USA: California, Mojave Desert
O. c. nelsoni	R	NA	AY904011	USA: California
O. c. nelsoni	S	NA	AY904012	USA: California, Transverse Ranges
O. c. nelsoni	V	NA	AY904015	USA: California
O. c. nelsoni	X	NA	AY904017	USA: California
O. c. nelsoni	CP1	NA	KU363640	USA: Arizona, Colorado Plateau
O. c. nelsoni	CP1.2	NA	KU363641	USA: Arizona, Colorado Plateau
O. c. nelsoni	CP2	NA	KU363642	USA: Arizona, Colorado Plateau
O. c. nelsoni	CP3	NA	KU363643	USA: Arizona, Colorado Plateau
O. c. nelsoni	CP4	NA	KU363644	USA: Arizona, Colorado Plateau
O. c. nelsoni	CP5	NA	KU363645	USA: Arizona, Colorado Plateau
O. c. mexicana/nelsoni	CS1	NA	KU363646	USA: Arizona, Colorado Plateau/USA: Arizona, Sonoran Desert/USA: New Mexico, Chihuahuan Desert
O. c. mexicana/nelsoni	CS2	NA	KU363647	USA: Arizona, Colorado Plateau/USA: Arizona, Sonoran Desert
O. c. mexicana/nelsoni	CS2.2	NA	KU363648	USA: Arizona, Colorado Plateau/USA: Arizona, Sonoran Desert
O. c. nelsoni	GC1	NA	KU363649	USA: Arizona, Colorado Plateau/USA: Nevada, Great Basin
O. c. nelsoni	GC1.2	NA	KU363650	USA: Arizona, Colorado Plateau/USA: Nevada, Great Basin

Taxonomic Identification	Sample ID	*Cyt*b	D-loop	Locality
O. c. nelsoni	MG1	NA	KU363651	USA: California, Mojave Desert
O. c. nelsoni	MG2	NA	KU363652	USA: California, Mojave Desert
O. c. nelsoni	MG3	NA	KU363653	USA: California, Mojave Desert
O. c. nelsoni	MG4	NA	KU363654	USA: California, Mojave Desert
O. c. nelsoni	MG5	NA	KU363655	USA: California, Mojave Desert
O. c. nelsoni	MG6	NA	KU363656	USA: California, Mojave Desert
O. c. nelsoni	MG7	NA	KU363657	USA: Nevada, Great Basin
O. c. nelsoni	MG9	NA	KU363658	USA: Nevada, Great Basin
O. c. nelsoni	MG10	NA	KU363659	USA: Nevada, Great Basin
O. c. sierrae	SN1	NA	KU363690	USA: California, Sierra Nevada Range
O. c. texianus	TK260051	OQ692329	OQ725120	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260052	NA	OQ725121	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260053	NA	OQ725122	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260054	NA	OQ725123	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260055	NA	OQ725124	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260056	NA	OQ725125	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260059	OQ692330	OQ725126	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260060	OQ692331	OQ725127	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260061	NA	OQ725128	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260062	OQ692332	OQ725129	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260063	OQ692333	OQ725130	USA: Texas, mountains surrounding the Van Horn area
O. c. texianus	TK260064	OQ692334	NA	USA: Texas, mountains surrounding the Van Horn area
O. c. mexicana/nelsoni	TK260075	NA	OQ725131	USA: Arizona or Nevada
O. c. mexicana/nelsoni	TK260076	NA	OQ725132	USA: Arizona or Nevada
O. c. mexicana	TK260077	OQ692335	OQ725133	USA: New Mexico: Guadalupe Mountains
O. c. texianus	USNM110047	NA	OQ725135	USA: Texas, Guadalupe Mountains, Mckitterick Canyon
O. c. texianus	USNM110342	NA	OQ725136	USA: Texas, Guadalupe Mountains, Mckitterick Canyon
O. c. texianus	USNM110388	NA	OQ725137	USA: Texas, Guadalupe Mountains, Mckitterick Canyon, Near

Taxonomic Identification	Sample ID	*Cytb*	D-loop	Locality
O. c. texianus	USNM118256	NA	OQ725138	USA: Texas, Guadalupe Mountains, S Of Guadalupe Peak
O. c. texianus	USNM118257	NA	OQ725139	USA: Texas, Van Horn, Mountains North Of
O. c. texianus	Barrow_Museum_84.877.1	NA	OQ725140	USA: Texas, Beach Mountains
O. c. texianus	Barrow_Museum_84.877.2	NA	OQ725141	USA: Texas, Beach Mountains
O. c. texianus	Barrow_Museum_84.877.3	NA	OQ725142	USA: Texas, Beach Mountains
O. c. texianus	GUMO-00711	OQ692336	OQ725134	USA: Texas, Guadalupe Mountains National Park
O. c. weemsi	NA	NA	AY116623	Mexico: Baja California Sur

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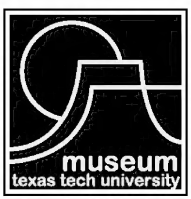

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